INTERAGENCY RESEARCH ANIMAL COMMITTEE (IRAC)

RECOMMENDATION ON LD_{50} TESTING

RECOMMENDATION

The LD_{50} test evaluates acute lethality from exposure to a substance or product. An LD_{50} value is the dose at which 50 percent of the test animals can be expected to die. This is used to: A) classify substances or products for regulatory purposes including safe transportation and labeling, B) provide information for treatment of acute intoxications, C) standardize certain biological products, D) set dose levels for subsequent toxicity studies, E) provide comparative information on the chemical's dose response curve, and F) provide data for evaluation and validation of alternative test methods. The LD_{50} tests have become controversial among toxicologists, animal welfare organizations, legislators and the public primarily due to the ethics of using a large number of animals and evaluating only mortality. IRAC studied this issue in depth and recommends the following:

1. The **Classical LD_{50}** test should only be conducted when specifically justified for reasons of scientific necessity and approved by the Institutional Animal Care and Use Committee.

2. Toxicity testing procedures based on the principles of **reduction** and **refinement** (e.g., Limit Test, etc.) should be used until alternative test methods become validated.

BACKGROUND

IRAC has reviewed the policies and recommendations of Federal Agencies and international organizations relevant to the **LD_{50}** test. These organizations include the Environmental Protection Agency, the Food and Drug Administration, the Consumer Product Safety Commission, the Department of Transportation, the National Toxicology Program[1], the Organization for Economic Cooperation andDevelopment, the British Toxicology Society, and European Chemical Industry Ecology and Toxicology Center. There is consistency among the policies and recommendations on the following points:

1. The use of the **Classical LD_{50}** test, which may require the use of 100 or more animals to establish the desired statistical confidence limits and evaluates only mortality, is unnecessary for the determination of acute oral toxicity.

---

[1] The National Toxicology Program includes the National Institute of Environmental Health Sciences of the NIH, the National Center or Toxicological Research of the FDA, and the National Institute for Occupational Health and Safety of the CDC.

2. Tests involving animals are currently essential in the determination of acute oral toxicity, which is a step in the assessment of the potential hazard of a chemical or product.

3. There are recommended alternatives to the **Classical LD_{50}** test utilizing the principles of **reduction** and **refinement** (see attached - Summary of Current Policies).

4. A validated **in-vitro** test or battery of tests is presently not available that can be used as a replacement for tests on animals in the determination of the **LD_{50}** dose. There are tests in various stages of development and/or validation. Instead of waiting for validated test(s) of the universe of chemicals or products, the process of development and/or validation may be accelerated using a group of related chemicals or products. For that group, validated test(s) could be accepted as a screen or as a replacement of the **LD_{50}** test on animals.

DEFINITIONS

1. **Classical LD_{50}**

   The Classical LD_{50} test is used to determine the lethal dose (LD_{50}) of a substance that will kill 50% of test animals. Typically, this method can use 100 or more animals. The test material is administered in increasing doses, usually 5 or more, to groups of 10 male and 10 female animals. Mortalities are recorded within a given period, and the LD_{50} is determined with the aid of statistical calculations.

2. **Limit Test**

   The limit test is used to determine if the toxicity of a test substance is above or below a specified dose. Five to ten animals of each sex or 10 animals of the susceptible sex are administered a dose specified by regulations. Toxic responses occurring within a given period are recorded. Based on the results, a regulatory action or additional testing may be required.

SUMMARY OF CURRENT POLICIES

U.S. ORGANIZATIONS

A. Consumer Product Safety Commission:

- Strongly discourages **Classical LD_{50} test**.
- Recommends other alternatives - existing animal data, prior human experience, expert opinion.
- Recommends the Limit test.

B. Department of Transportation:

- Discourages the use of the **Classical LD_{50} test**.
- Recommends the use of existing animal data and human experience.
- Recommends the Limit test.

C. Environmental Protection Agency:

- Discourages the use of the **Classical LD_{50} test**.
- Uses Structurally Related Activities (SAR) to obviate the need for testing on animals.
- Recommends the Limit test.
- When testing beyond the Limit test is conducted, recommends use of abbreviated test methods such as the approximate lethal dose, the moving average, and the up-and-down methods. Stresses the need for multiple end point evaluations, including onset, nature, reversibility of effects and gross necropsy.

D. Food and Drug Administration:

- Does not require the use of the **Classical LD_{50} test**.
- Accepts alternatives.
- Refers to the Limit test.

E. National Toxicology Program:

- Does not use **Classical LD_{50} test**.
- Uses in-depth toxicology with many end points.

INTERNATIONAL ORGANIZATIONS

A. Organization for Economic Cooperation and Development:

- Discourages the use of **Classical LD_{50} test**.

- Recommends the Limit test (2 g/kg dose).
- When compound related mortality occurs in the limit test, then 5 animals per dose, at least 3 dose levels are used to produce a range of toxic effects and mortality rates; clinical observations and pathological investigations are conducted.
- A fixed dose procedure, which uses morbidity instead of mortality as the end point, is also recommended.

B. British Toxicology Society:

- The LD_{50} should only be determined with any accuracy where scientifically and ethically justified. Such cases are relatively rare.
- Examination of few animals in detail rather than many for statistical purposes.
- Limit tests could be used, provided animals in distress are killed humanely, if this would not interfere with the objectives.
- For classification of substances and preparations, a fixed-dose procedure targeted to acute signs could replace the current practice of LD_{50} determination.

C. European Chemical Industry Ecology and Toxicology Center:

- Acute toxicity profiles are important.
- **Classical LD_{50}** test is seldom necessary.
- Protocols exist for estimating lethal dose.
- LD_{50} above 2 g/kg is irrelevant.
- Regulations should not specify minimum number animals.
- Procedures for the selection of a toxicity class should be encouraged.
- Acute toxicity data may be unnecessary for the protection of human health from some products.
- Predictions based on alternatives as an aid to dose selection should be encouraged.

Attachment - Bibliography

BIBLIOGRAPHY - IRAC LD_{50} RECOMMENDATION

Alternative Methods in Toxicology. Vol 2. Acute Toxicity Testing: Alternative Approaches. Goldberg A. M. (Ed.). Mary Ann Liebert, Inc. Publishers, New York. pp. 292.

British Toxicology Society, (1984). A new approach to the classification of substances and preparations on the basis of acute toxicity. Human Toxicology 3:85-92.

Consumer Product Safety Commission, (1984). Animal Testing Policy, Federal Register 49:22522-22523.

Department of Transportation, (1992). Poisonous material. Class 6, Division 6.1-Definitions. 49 CFR Section 173.132.

European Chemical Industry Ecology and Toxicology Center, (l985). Acute toxicity tests, LD_{50}(LC_{50}) determinations and alternatives. Monograph 6, Brussels, Belgium.

Environmental Protection Agency, (1988). Revised policy for acute toxicity testing. Office of Pesticides and Toxic Substances, September 22, 1988.

Food and Drug Administration, (1988). LD_{50} Test Policy. Federal Register 53:39650-39651.

Organization for Economic Cooperation and Development, (1987). Guidelines for testing of chemicals. Section 4: Health Effects. Test Guideline No. 401. Acute Oral Toxicity. Paris, France.

Organization for Economic Cooperation and Development, (1992). Guidelines for testing of chemicals. Section 4: Health Effects. Addendum to Test Guideline 401. Fixed Dose Procedure. Paris, France.

SOT Position Paper - Comments on the LD_{50} and Acute Eye and Skin Irritation Tests, (1989). Fundam. Appl. Toxicol. 13:621-623.

IRAC - 10/25/89, Revised 9/22/93

ARAC - Adopted Original 9/30/92, Adopted Revision 11/10/93

ARAC - Reapproved - 5/8/96.